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(54) Microbiological testing

(57) Apparatus for performing a plurality of microbiological tests comprises a layer of agar or other nutrient gel filling a plurality of channels (2) which communicate with a common region (3 and 4) from which the apparatus may be charged with e.g. liquid agar prior to use. Channels (2) have e.g. wells (1) for test substances and indicia (7) to permit a direct visual measurement of the effect of a test substance on the growth of a microorganism on said agar, when said test substance has been allowed to diffuse along said channel from a determined point of application to form a concentration gradient of said substance along said channel. Test substances in wells (1) are e.g. antibiotics or vitamins.

FIG. 1.

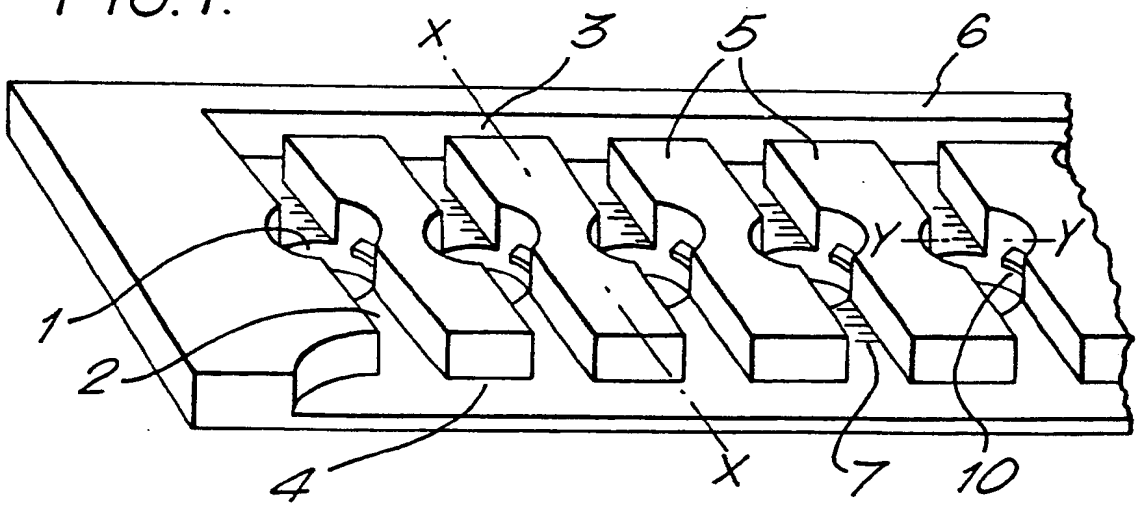

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FIG. 1.

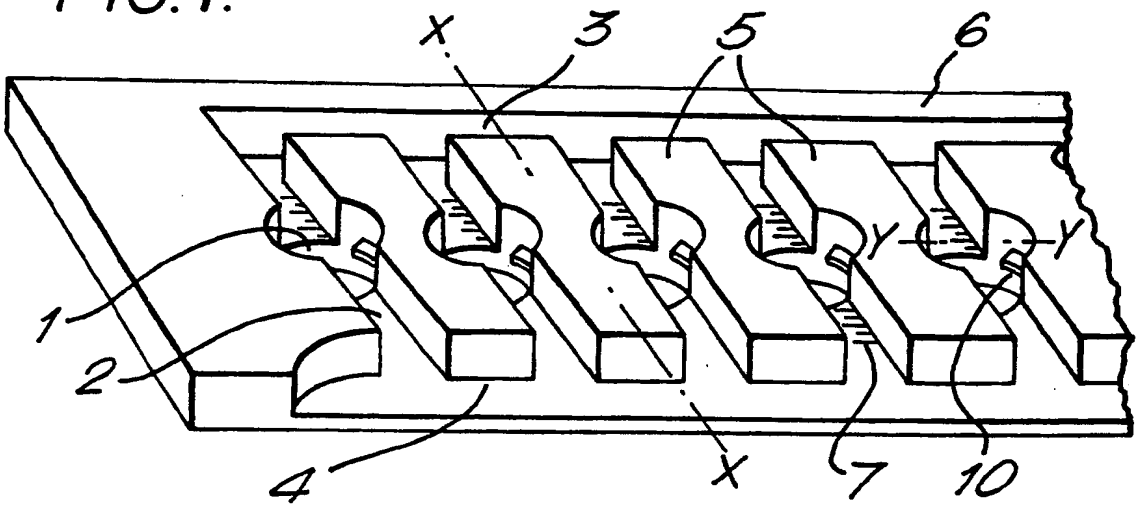


FIG. 2.

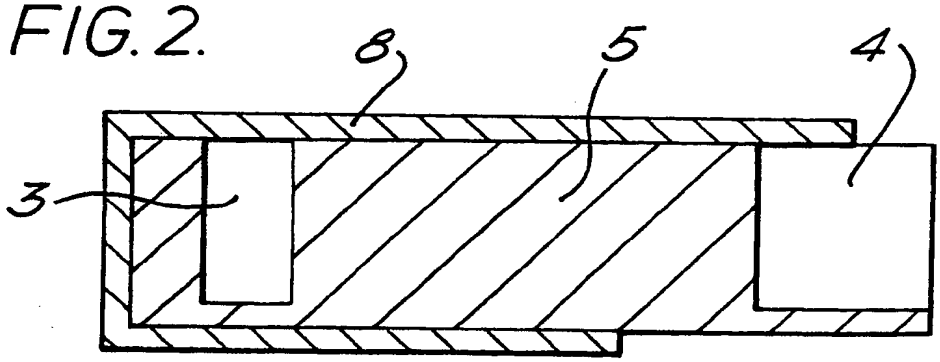


FIG. 3.

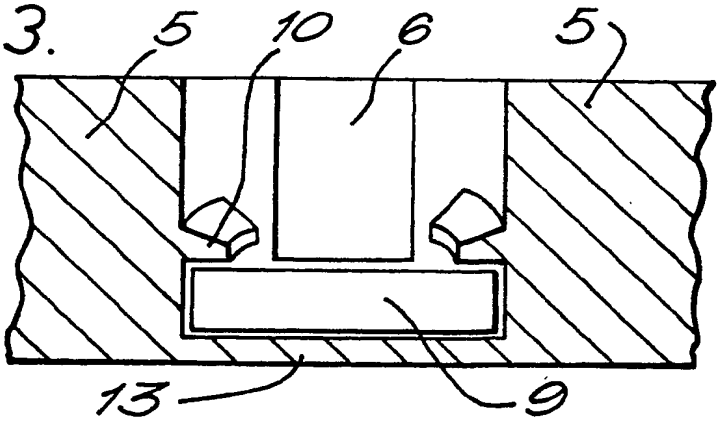


FIG.4.

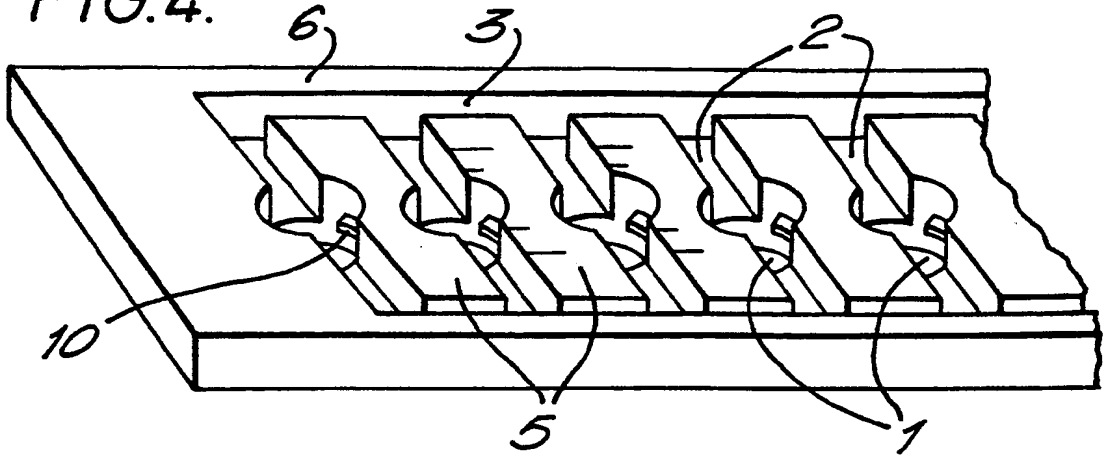


FIG.5.

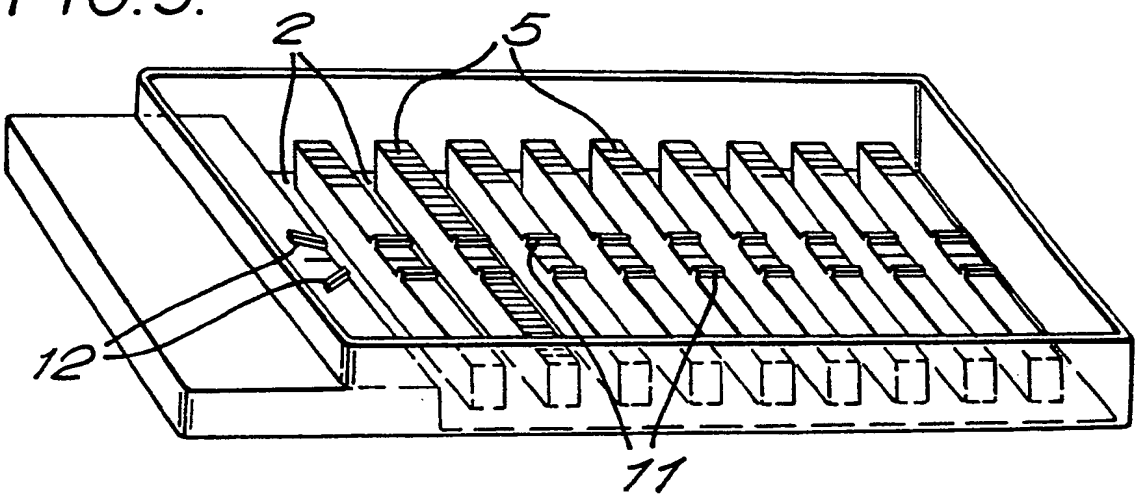


FIG.6.

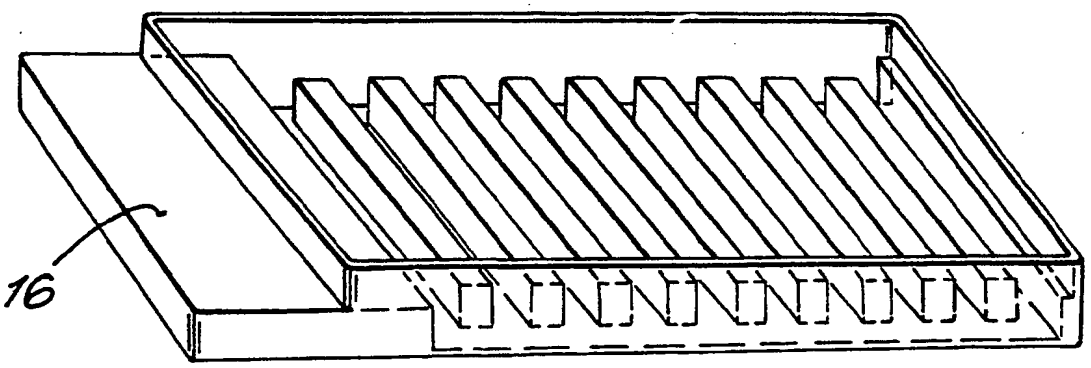


FIG. 7.

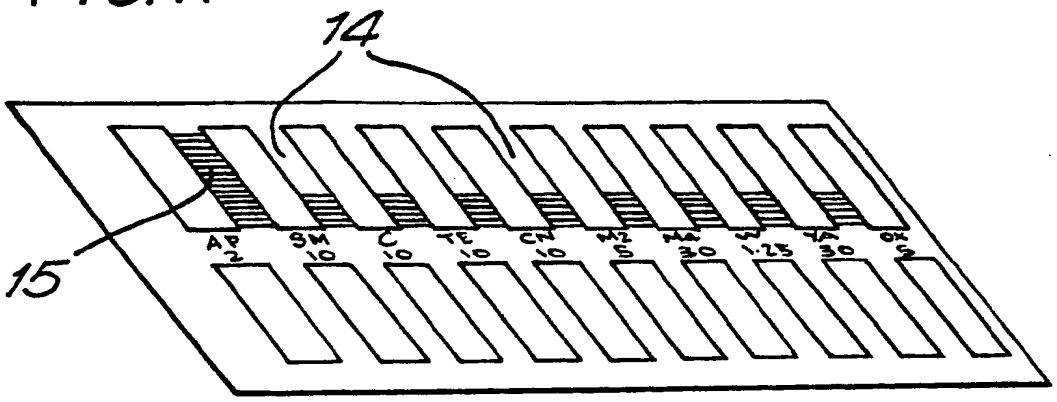


FIG. 8.

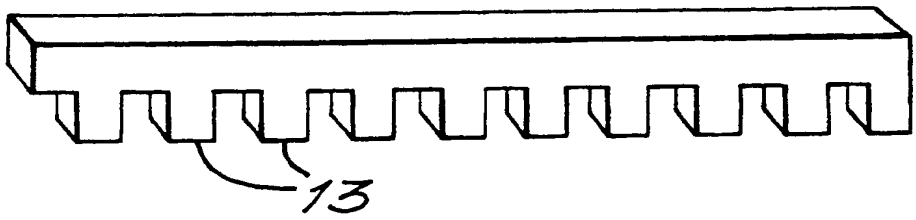
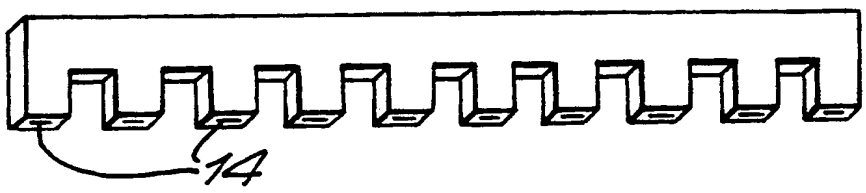


FIG. 9.



"Apparatus for microbiological testing"

The invention relates to apparatus for microbiological testing, e.g. for assaying microbial sensitivity to one or more antibiotics.

It is known to test antibiotic sensitivity by measuring zones of inhibition of microbial growth on an agar plate. In this known method, agar, or other nutrient medium, is poured into a container such as a Petri dish and allowed to solidify, to produce an uninterrupted plate of agar. The surface of the agar is then inoculated with the microorganism under test, and paper discs, each impregnated with an antibiotic are applied to the surface. The plate of agar is incubated, whilst keeping the surface of the agar horizontal, until zones of inhibition of microbial growth form. During the incubation period, the antibiotic diffuses through the medium so that a concentration gradient, which is a function of the amount of antibiotic on the paper disc, is established in the medium. Zones of inhibition form in regions where the antibiotic is present in sufficient concentration to prevent microbial growth. In these regions, the medium remains clear, whereas microbial growth produces turbidity in or on the medium.

With conventional methods for testing antibiotic sensitivity, zone sizes are measured and compared to sizes obtained for known sensitive and resistant strains and zone size limits are set enabling classification of the organism as "sensitive", "resistant" or "intermediate".

However, if more than one test is carried out on the same plate of agar, interference can occur between the separate tests. Unless inconveniently large plates of agar are used, antibiotics in separate tests can diffuse from

different paper discs into the same region and produce overlapping zones of inhibition. Only six antibiotics may conveniently be tested on the standard 9 cm diameter Petri plate.

A further disadvantage of this method is the need for measurement and comparison or calculation before a result can be obtained. Such extra handling is time-consuming, open to error and increases the risk of the operator coming into contact with dangerous pathogens.

Alternative methods of testing antibiotic sensitivity currently in use include measurement of the electrical conductance or impedance of cultures grown in the presence of the drug, or of the turbidity generated in such cultures. These values are then compared with those of a culture which has already been classified. Again, these methods require a certain amount of calculation and comparison usually requiring continuous read-out by expensive equipment.

Such methods may also involve tests on a multiplicity of media containing different concentrations or combinations of the antibiotic, and are thus time-consuming to set up and prone to errors.

According to the invention, there is provided apparatus for performing a plurality of microbiological tests, said apparatus comprising a plurality of channels which communicate with a common region from which the apparatus may be charged with liquid agar or other nutrient gel prior to use; characterised in that indicia are provided adjacent said channels to permit a direct visual measurement of the effect of a test substance on the growth of a microorganism on said agar, when said test substance has been allowed to diffuse along said channel from a determined point

of application, to form a concentration gradient of said substance along said channel.

It is evident that the apparatus may be used for any kind of assay or test which requires the measurement of a zone of inhibition or activation of microbial growth. Thus, the substance under test may either inhibit growth (e.g. antibiotics), or promote it (e.g. vitamins). For use in assays, the concentration of an antibiotic in a sample, for example, may be determined by comparing the zone of inhibition produced by a known amount of the solution of unknown concentration with those produced by known amounts of standard solutions of the antibiotic, or by the dehydrated solids from these volumes and concentrations.

In a preferred embodiment, the channels are parallel, communicating with a common region at one or both ends. Said common region may communicate with parallel channels on either side thereof, thereby permitting a greater number of tests to be performed, e.g. at two different concentrations of antibiotics, to determine sensitivity break-point values. If desired, said common region may be open-sided, whereby the apparatus may be charged with liquid agar after closing over said test regions with a removable cover and tilting the apparatus so that said common region is uppermost.

Other configurations, e.g. channels extending radially from a central common region, are also feasible.

The substance or substances under test can be applied to the channels using conventional methods e.g. by applying to the surface paper discs impregnated with the substance. Preferred forms of apparatus, however, possess means for facilitating the application of test substances to

the channels, e.g. by locating a carrier on which the test substance or substances are impregnated.

In one such preferred form of apparatus, each channel comprises a depressed portion forming a well. The wells, which conveniently are centrally disposed in the test regions, may be of any convenient shape or size, but they are preferably provided with means adapted to retain a disc or other carrier impregnated with the substance under test. Such means may e.g. be an overhanging rim, lip or projection beneath which the carrier may be trapped, or a protrusion from the bottom of the well on which the carrier may be impaled, e.g. a conical peg designed to locate in a suitably placed aperture in the carrier. It is also possible to retain the carrier in the well by adhesive means.

Using this form of apparatus, a known quantity of test substance may be applied to the disc using conventional methods. The disc is then inserted into the well before the molten medium is charged to the apparatus.

It is preferred that the discs or other carriers bearing the test substances or standard samples for use in assays be inserted in the apparatus prior to hermetically packaging said apparatus e.g. in plastics film or foil. The whole apparatus may then be sterilised, e.g. by X-rays or gamma radiation, and stored in a sterile condition until required for use.

In another embodiment of the invention, the wells are not provided with carriers for the test substances. Instead, the aforementioned cover plate, or a separate well-forming member, carries projecting portions, preferably of truncated pyramidal or truncated conical form, which align with the wells and cause a void to be left therein when the apparatus is charged with nutrient

medium. On removal of the cover plate or well-forming member, the voids in each well may be filled with solutions of test substances by means of a micropipette or the like.

Alternatively, a test substance applicator may comprise projections, e.g. analogous to the teeth of a comb, dimensioned to align with the wells previously formed in the nutrient medium. Each tooth is provided with an inlet e.g. in the form of a bore or slot designed to draw up a standard volume of liquid by capillarity. The applicator may then be charged from reservoirs of standard concentrations of test substances, and may be used to transfer determined volumes to the respective wells.

In another embodiment, the test substance or substances are applied from a carrier strip placed across said channels, means being provided to locate such strip in a determined position, e.g. by lugs, projections, groove or slot.

It is preferred to apply the test substances to a central point in each channel so that they cannot diffuse through said common region and also so that large zones of inhibition are able to form.

The size of the zone of inhibition or activation may be estimated visually by inspection against the indicia provided adjacent said channel. It is preferred to provide a fixed scale marked on the apparatus itself. Each channel may be calibrated so that e.g. the sensitivity of a microorganism to the antibiotic under test can be read directly from the apparatus. Thus, in contrast with conventional methods, once the apparatus has been calibrated there is no need for any measurement, calculation or comparison.

Alternatively, the indicia may be provided on a carrier, e.g. of paper or other sheet

material, to which the test substance or substances have been applied.

Generally the apparatus will be made of a transparent or translucent material, e.g. glass or synthetic resin, so that it may be trans-illuminated for assessment of the tests.

The invention will now be described with reference to the accompanying drawings, which are by way of example only.

Figure 1 shows a partial perspective view of one embodiment of our invention.

Figure 2 shows a cross-section through the apparatus of Figure 1, together with the cover (8) in position. The cross section is taken through the line X-X shown in Figure 1.

Figure 3 shows a cross-section through one of the wells (1) shown in Figure 1. The section is taken through the line Y-Y.

Figures 4, 5 and 6 show partial perspective views of three further embodiments of our invention.

Figure 7 is a perspective view of a test substance carrier for use with the embodiment of Figure 6.

Figure 8 is a perspective view of a well-forming member for use with the apparatus of Figure 5; and

Figure 9 is a perspective view of a test substance applicator for use with the apparatus of Figure 5.

The apparatus of Figure 1 possesses a plurality of test regions (2) in the form of parallel channels, communicating with common regions (3) and (4). Each test region is provided with a well (1) and a scale (7) on the base of each channel. This scale may alternatively be on the surface of the separating walls (5). The test regions are separated by walls (5), and a

containing wall (6) retains nutrient medium (not shown) within the apparatus. The apparatus is moulded from glass or synthetic resin.

The cover (8) shown in Figure 2 has a planar lower surface and rests in intimate contact with the top surface of the container and separating walls. The apparatus is open-sided and part of the communicating channel (4) is uncovered so that the apparatus may be charged with liquid agar when the cover is in position. The cover may be provided with narrow grooves aligned with the walls (5) so that air can escape from the apparatus. Using this form of apparatus, each test region is completely filled to the height of the separating and container walls, without the inclusion of air.

The well shown in cross-section in Figure 3 houses a paper (or other porous) disc (9) retained by projections (10). If desired the closure of the well may be performed by the disc (9) and the wall (13) may be omitted.

The apparatus shown in Figure 4 possesses all four container walls. After charging with liquid agar into common region (3), the apparatus must be held horizontally so that the agar is uniform in depth when set.

The apparatus shown in Figure 5, may be charged with test substance in a number of different ways. In one embodiment, the test substance is applied to the surface of the agar. A paper strip (not shown) is first impregnated with the test substances and then laid across the apparatus, at right angles to the test zones. The paper is held in position by the projections (11). To prevent diffusion of the test substances through the paper, the regions containing the test substances may be separated by hydrophobic regions

impregnated with an impenetrable substance such as PVC or silicone or other suitable compound.

The lugs (12) are arranged to cooperate with a suitably-shaped end portion of the paper strip so that only a correctly-shaped and impregnated strip is usable with the apparatus. It is envisaged that a number of types of apparatus be provided, e.g. for testing antibiotics against different main classes of pathogen - e.g. Gram positive, Gram negative or urological organisms. Each apparatus could be designed to accommodate a uniquely-shaped paper strip impregnated with suitable antibiotics for testing the organisms concerned and may be marked with the appropriate inhibition zone sizes to enable the grading "sensitive", "resistant" or "intermediate" to be made, or to enable minimum inhibitory concentrations (MIC values) to be calculated.

For a particular drug the MIC value corresponding to any particular zone size can be derived from a regression line analysis of the plots of $\log_2$ MIC, obtained by a standard tube dilution method, versus zone diameter in millimeters, derived by an agar diffusion method under equivalent conditions, performed for a large number (several hundred) of different bacterial cultures. Such regression line data already exist for a large number of antibiotics in common use and form the basis of the decisions of disc drug potencies for the performance of standard tests. Alternatively, the device itself could be used to obtain the zone diameters appropriate to the MIC values performed at the same time.

In a preferred embodiment for the performance of microbiological assays, a plate of the type shown in Fig.5 is charged with molten agar containing the assay organism and a well-forming "comb" (Fig.8) comprising frusto-pyramidal "teeth"

is inserted into the plate such that the "teeth" of the comb are inserted into the mid-positions of the channels by locating the spine of the well-former exactly between the lugs (11, Fig.5), the width of the spine being the size of the inter-lug space.

When the agar has set the well-former is removed carefully. To assist easy removal the protrusions which form the wells may be of truncated pyramidal shape, or of truncated conical shape.

Each well so formed is then charged with a standard volume of solution of the antibiotic or other substance under test so as to form a suitably graduated concentration series in the majority of the wells. One or more wells are reserved for the test samples, being unknown concentrations of the antibiotic.

After incubation of the plate the sizes of the zones of inhibition for each known concentration may be read directly from the plate and used to plot a standard curve of log concentration vs. zone diameter. The concentration of the unknown solution may be obtained by interpolation of its zone diameter into the standard curve.

Alternatively it may be sufficient simply to read directly the zone diameter of the unknown, and to compare this directly with the zone diameter of standard concentration nearest to the unknown. Such an estimate, using the appropriate graduated series of standards (e.g. 2mg/1 concentration differences for gentamicin), would give adequate evaluations of serum concentration for the monitoring of most clinical dosage regimes.

A simplified method of establishing the concentration gradient series of drug standards

may be effected by means of a capillary comb, Fig.9. In this comb there is a slot in the centre of each "tooth" (14) of such dimension as to draw into each "tooth" a standard volume of liquid by capillary absorption. Such a comb could be charged from reservoirs of appropriate standard concentrations and used directly (by hanging the comb in the plate wells each of which has a standard volume of diluent already charged), or the comb may be dehydrated and stored (under appropriate conditions) for future use.

Similar methodology could be used for the assay of vitamins, e.g. folic acid in samples except that there would be growth only in the areas proximate to the vitamin source, related in zone size to the concentration of the vitamin source.

The teeth of a capillary comb could be of round or rectangular shape, the prime consideration being that each lumen should draw up the same volume of liquid.

An alternative to a capillary comb would be an absorbent paper comb but with an impervious hydrophobic spine. Each tooth would contain an absorbed amount of dehydrated active agent such that on immersion in standard volumes of suitable liquid in the wells, the required concentration of agent would be established.

In an alternative application of the method the lugs (11, 12) in Fig. 5 may be omitted from the design and the drug-bearing paper strips may be centrally located in a plate of the type shown in Fig. 6, using a paper template as shown in Fig. 7. In this template the cross connecting strips (14) are printed with the scale (15) for measuring the zone sizes. The said cross strips in this application are water resistant and rest upon the tops of the walls (5).

In both latter embodiments the carrier for the test substance or substances may be held in contact with the agar in the channels by means of projections of appropriate length descending from the underside of a lid which covers the entire agar area of the plate.

In order to make the zone edge more obvious, and to do so at an earlier time, an indicator may be applied as a solution by rapidly flooding the plate and removing the excess. The layer of cells outside the zone would be stained, leaving the inhibition zone unstained. This could be achieved by the use of an appropriate oxidation-reduction indicator since all growing bacterial cells lower the redox potential. Alternatively a fluorescent dye might be used, or any other suitable means.

For reading the MIC value from zone sizes, nomographs may be provided in the form of a printed transfer on the lid.

All the applications mentioned can be readily automated by the use of an optical scanner capable of detecting opacity differences and the signals can then be processed to yield the sensitivity classification of the organism for each drug, the MIC value, the dosage required in clinical use and the cost of treatment; all controlled by means of suitable computer software, and printed in a hard copy format most suited to the users needs. Additionally the data stored could be computer analysed at any time to give the epidemiological progress of drug-resistant strains in the local community, and by combination with data from other sources, the national picture.

It is additionally convenient if the specific test is also automatically identified. This may be achieved by a bar code strip on the panel (16) which was also scanned. The said panel

may incorporate the relevant patient and specimen data.

A device of the type shown in Fig. 6 could be used in a number of additional different applications. For example it could be used to facilitate the phage-typing of bacteria. For this purpose the cross strips of paper would be designed to contact the agar surface in the channels, but preferably only half of each channel so that the other half would serve as a control of normal growth. In use each paper arm would be impregnated with dried phage particles comprising the number used in a typing set, using one phage strain per arm. The phage template would then be impacted onto the inoculated plate, then removed and the plate incubated to reveal the subsequent formation of phage plaques. The pattern of positive and negative effects within the typing set determines the phage type.

In another application, we have designed a system for the identification of bacterial species based on their biochemical reactions. The substrates to be modified by the organism are dried onto a paper template similar to the one used for phage-typing. Each substrate area is isolated from the next by a hydrophobic barrier, and the substrate-bearing arms are left in contact with the inoculated agar surface once the strip has been applied. The substrates diffuse along the agar channels, and are converted by the growing organisms. The strips also include where possible an indicator which directly indicates the nature of the change wrought by the organism; for example, a lactose substrate including Andrade's indicator would turn pink on fermentation of the lactose with formation of acid products. The tests for identifying members of the Enterobacteriaceae might include the following

substrates: glucose, lactose, mannitol, sorbitol, inositol, melibiose, saccharose, rhamnose, arabinose, amygdalin, arginine, ornithine, lysine, sodium citrate, urea and tryptophan. Other changes resulting from metabolism of the growing bacteria may be tested by adding reagents to the test strips after a period of growth. Such tests may include addition of Kovacs reagent to show indole production, alpha-naphthol and potassium hydroxide for acetoin production, tetramethyl p-phenylenediamine to indicate the presence of cytochrome oxidase, Greiss reagent to indicate nitrate reduction, hydrogen peroxide to demonstrate catalase, etc.

It has been possible to establish the dimensions required for the channels (2) in the various applications. For sensitivity testing by the paper strip method, experiments have been performed to determine the zone size by a standard method for single drugs and standard strains of organism. The conditions required to produce the same zone size with the same organism and the same drug disc on agar strips are then established. As an example, a standard method using Iso-sensitest agar (Oxoid) with a 30 mcg Cephradine disc and Escherichia coli NCTC 10418 gave a zone diameter of 18 mm. Using the same batch of discs the zone diameters obtained with the same organism inoculated to the same extent on the same agar but in strips of varying depths and widths was determined.

By interpolation it was possible to state the parameters for agar strip diffusion which will yield the same size of zone. These are: 7 mm wide by 5.4 mm deep, or 10 mm wide by 4.1 mm deep. Using apparatus which gives equivalent sized zones for agar strip (channels) diffusion, means that

the standards already established for determining "resistant", "intermediate" and "sensitive" classification will be directly applicable. Similar experimentation can be used to determine the parameters for a device to utilise the standards established for the Kirby-Bauer technique, as given by the National Committee for Clinical Laboratory Standards for use in the United States.

Claims:

1. Apparatus for performing a plurality of microbiological tests, said apparatus comprising a plurality of channels which communicate with a common region from which the apparatus may be charged with liquid agar or other nutrient gel prior to use; characterised in

that indicia are provided adjacent said channels to permit a direct visual measurement of the effect of a test substance on the growth of a microorganism on said agar, when said test substance has been allowed to diffuse along said channel from a determined point of application to form a concentration gradient of said substance along said channel.

2. Apparatus of claim 1 wherein said channels are parallel and communicate with common regions at both ends.

3. Apparatus of claim 1 or 2 comprising means for locating a carrier on which the test substance or substances are absorbed.

4. Apparatus of claim 3 comprising means for locating in a determined position an absorbent strip placed across said channels, the test substance or substances being carried on said strip.

5. Apparatus of claim 3 wherein each channel comprises a depressed portion forming a well, the well being provided with means adapted to retain an absorbent disc or other carrier impregnated with the substance under test.

6. Apparatus of claim 5 wherein said discs or other carriers bearing the test substances or standard samples for use in arrays have been inserted in said apparatus prior to hermetically packaging and sterilising said apparatus.

7. Apparatus according to claims 1 or 2 in combination with a removable well-forming member having projections round which said agar or other nutrient gel may be cast.

8. Apparatus according to claim 7 in combination with a test substance applicator comprising projections designed to align with the wells previously formed in said agar or other nutrient gel, each projection being provided with an inlet designed to draw up a standard volume of liquid by capillarity.

9. Apparatus according to any of the preceding claims wherein said indicia are provided on a fixed scale on the apparatus.

10. Apparatus according to any of claims 1 to 4 wherein said indicia are provided on a carrier of absorbent sheet material to which the test substance or substances have been applied.

11. Apparatus according to any of the preceding claims wherein said indicia are characterised by marked inhibition zone limits corresponding to gradings of "sensitive" and "resistant" for a given antibiotic.

12. Apparatus according to claim 1 substantially as herein described.

13. Apparatus substantially as illustrated in the accompanying drawings.

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